ELECTRONIC SOMATIC CELL COUNT

Somascope MKII

(Unless otherwise stated all tolerances ±5%)

1. Laboratory requirements (see CP, item 33 & 34) _____

   a. Un-preserved samples may be run up to 72 hours after initial collection _____

   b. Samples may be run up to 7 days after initial collection if preserved with 0.02% 2-bromo-2-nitropropane-1,3-diol (Bronopol™) or 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

   c. Comparative test with DMSCC _____

      1. Performed by each analyst performing ESCC test _____

      2. Test 4 samples (100K-200K, 300K-500K, 600K-800K and 900K-1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC (three separate sub-samples each, do not read same sample three times) _____

      3. Results must be shown to be acceptable prior to official testing by analyst performing comparison, i.e. analyst is not certified until found acceptable. **(co-requisite for certification)** _____

      4. Copy of comparison and results in QC record (or easily accessible file in laboratory) _____

   d. Analysts certified in DMSCC _____

APPARATUS

2. Cultural procedures, items 1-5 _____

3. Electronic Somatic Cell Counter _____

   a. Somascope MKII manual _____ _____

   b. Somascope MKII automatic _____ _____

4. Water bath _____

   a. Circulating and thermostatically controlled to 37-42C _____

REAGENTS

5. Staining Kits _____

Warning: Before using the chemicals for making up the staining solution, read the Material Safety Data Sheet (MSDS) provided in the appendix of the manual, wear safety glasses and latex gloves when handling the chemicals

a. One liter concentrate kit, makes 5 L staining solution when diluted mixed with deionized (DI) or MS water _____

Date Rcd _____ Exp. Date _____ _____

1. Store at 0-5C and protected from light _____

b. Twenty liter powder kit, consists of three different components, mixed with 20 L DI or MS water _____

Date Rcd _____ Exp. Date _____ _____

1. Staining Concentrate (powder) _____

a. Store powder 0 - 5C and protected from light _____

2. Staining Detergent (liquid) _____

a. Store at room-temperature protected from light _____

3. Staining Buffer (powder) _____

a. Store at room-temperature protected from light _____

WORKING SOLUTIONS

6. Working Staining Solutions _____

a. One liter Concentrate Kit _____

1. Add 4 L of DI or MS water _____

2. Mix on a magnetic stirrer at room temperature to make working stain solution _____

b. Twenty liter Powder Kit

1. Dissolve the staining buffer (item 5b3) in about 18 L of DI or MS water and stir until the powder is fully dissolved _____

2. Add the Staining Detergent (item 5b2) to about one liter of warm (35 - 45C) DI or MS water and mix well (preferably with a magnetic stirrer) to dissolve the detergent _____

a. The detergent must be well dissolved, no powder residue visible (optical test) _____

3. Add the detergent solution (item 6b2) to the 18 L of staining buffer (item 6b1) and mix _____

4. Dissolve the Staining Concentrate (item 5.b.1.) in 3 mL of 35-45C DI or MS water _____

a. Mix until the powder is dissolved (optical test) _____

b. Keep the concentrate (powder and solution) protected from strong light during preparation _____

5. Add the dissolved concentrate to the approximately 19 L of buffer and add DI or MS water until the total volume is 20 L _____

c. Store the working staining solutions up to 2 months at 0 - 5C protected from light _____

Date prep. _____ Exp. Date _____ _____

d. Use the staining solution at room-temperature _____

Date filled _____ _____

1. The contents of the 5 liter staining container can be left at room temperature _____

2. The contents must be used within 7 days _____

3. Clean container once a month as per manufacture instructions _____

7. Working Rinse Solution _____

a. Detergent Container _____

1. Alkaline detergent; DECON 90, Contrad 70 or RBS 50 _____

2. Fill the black detergent reservoir with about 50 mL of undiluted detergent in the Sample Preparation Unit _____

3. Check reservoir visually daily for volume and add more detergent as necessary _____

b. Water Container _____

1. Measure 5 mL of Triton X-100 and place in 100 mL of DI or MS water _____

2. Mix the above solution until the Triton X-100 is completely dissolved _____

3. Dilute the 100 mL mixture above to 5 liters with room temperature DI or MS water _____

4. Mix well _____

5. Pour the above into the water container provided with the instrument _____

START UP

8. Somatic Cell Counter _____

a. Check to see if the amount of staining solution (about 1.6 mL per sample), detergent and rinse solution (about 1 mL per sample) is of sufficient volume for the number of samples to be run _____

b. Solutions not be used beyond expiration date _____

c. Turn on the HBO lamp, Sample Preparation Unit and wait 15 minutes _____

d. Turn on the internal computer of the Somascope MKII _____

e. Put the peristaltic pump in operation position _____

f. Perform a clean sequence _____

g. Run at least 5 times with rinse solution (item 7b), last reading must be below 5 _____

h. **IF ANY PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** _____

i. Records maintained on all parameters each time instrument is used _____

9. Milk Standards _____

a. Commercially prepared: _____ _____
Lot# _____ Date Rcd. _____ _____

1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

2. Do DMSCC in triplicate on each standard in set and average counts, records maintained _____

3. DMSCC check performed in rotation by all certified analysts _____

4. Standards used within one week _____

b. Certified provider: _____

Lot# _____ Exp. Date _____ Date Rcd. _____ _____

1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

2. Maintain copies of all provided DMSCC values _____

3. Measure and maintain records of temperature (0-7.2C) of standards as received _____

4. Maintain copies of all correspondence regarding problems _____

5. Standards used by manufacturer's expiration date _____

c. Laboratory prepared (weekly) _____

1. Prepare from raw milk > 18 hours old preserved with 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

2. Or, preserved with 0.02% 2-brono-2-nitropropane-1,3-diol (Bronopol™) _____

3. Standards <u>cannot</u> be preserved with formalin _____

4. Prepare 4 standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M, use within one week
Date prep. _____ Exp. Date _____ _____

5. Do DMSCC in triplicate on each standard prepared and average counts, records maintained _____

6. DMSCC check performed in rotation by all certified analysts _____

d. Hourly Control Sample (instrument drift check) _____

1. Use one of the standards (items 11a or b) in the 500-800K range, run in triplicate and determine average _____

2. Optionally, prepare sufficient control/sample 500-800K range, run in triplicate and determine average _____

PROCEDURE

10. Testing Standards (each time instrument used) _____

    a. Heat standards to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature, used once and then discarded, i.e., do not re-use _____

    b. Mix by inverting at least 2x, test standards within 3 minutes _____

    c. Run the standards in triplicate and average the counts for each level, records maintained _____

    d. Each standard's average must be within 10% of the DMSCC (item 11) for that level, except within 15% for 100-200K standard, records maintained _____

    e. Repeatability - a standard in the 300K to 800K range must have a coefficient of variation (C_V) of 5% or less on 10 replicates, records maintained _____

    f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

11. Testing samples _____

    a. Heat samples to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature; samples must not be re-used and must be discarded after use _____

    b. Mix by inverting at least 2x, test samples within 3 minutes _____

    c. Samples must be tested within 10 minutes after being removed from waterbath _____

    d. Samples must not be reused and must be discarded after use _____

    e. Record number of cells counted for each sample _____

12. With continuous operation: _____

    a. Run a standard or optionally a control/sample (item 9d) in the 500K to 800K range hourly, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average) _____

    b. Run control 3x _____

    c. Run zero control (item 8g) hourly _____

d. Maintain records _____

13. Shut down procedure _____

a. Perform a clean cycle twice _____

b. Put instrument pipette in beaker of rinse solution _____

c. Release peristaltic pump _____

d. Switch off PC, SPU, internal computer of Somascope MKII, HBO-lamp in this order _____

14. Routine maintenance _____

a. Perform as described in operating manual _____

b. Maintain records _____

REPORTS

15. Computing and Reporting of Counts _____

a. Count obtained x 1000 is the cell count/mL milk _____

b. In reporting electronic somatic cell counts (ESCC/mL), record only first two left hand digits, raising second digit to next higher number when third digit is six or more _____

c. Report the two left hand digits (rounded) _____

1. If the third digit is 5 the second digit is rounded by the following rule _____

a. When second digit is odd round up, raising the second digit by 1 (odd up, 235 to 240) _____

b. When second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220) _____

d. If count on instrument is < 100 report count as < 100,000 ESCC/mL _____